PS04.01.26 CATALYTIC CONFORMATION OF PSEUDOMONAS 7A GLUTAMINASE-ASPARAGINASE (PGA): CRYSTAL STRUCTURE OF THE PGA-SO42-/NH4+ COMPLEX AT 1.7 Å RESOLUTION. C. Jakob1, M. LaCour2, K. Lewinski3, J. Roberts4, L. Lebioda2, J. Komorowski5, M. Hirokawa, T. Tanaka, T. Sands and Y. Mitsui, Department of BioEngineering, Nagasaki University of Technology, Nagasaki, Niigata, 940-21, Japan

Amidolyases from E. coli K12 and Erwinia chrysanthemi exhibit a relatively high specificity for asparagine and are referred to as asparaginases. The monoclinic crystal structure of L-Asparaginase II from E. coli K12 has been determined at 2.3 Å resolution by Swain et al. (1993). The asparaginase from E. coli A1-JKY3982 was used for crystallization in the present analysis. The molecule is composed of four subunits and the molecular weight is ca. 156 KDa. Three types of crystals, all belonging to a space group P2121, have been obtained, one of them as a hexameric complex (Asp-complex) and the other two as aspartic acid complexes (Asp-complex1 and Asp-complex2). The three types of crystals, the cell parameters were different from each other by up to 15%. In the Glu-complex the loop region in the active site is clearly resolved in the electron density map. Crystallographically there are two binding sites in each asymmetric unit. In the Asp-complexes, equivalent types of aspartate binding were observed for each of the two binding sites. This may be related to the presence of rigid loop region in each of the two binding sites. The crystal structure, in the Glu-complex, of the bound glutamate ligands was found to have a different conformation. Detailed analyses of the three types of crystals are in progress.


The ubiquitous calpains have a precise physiological role is uncertain, however it is likely that they are involved in cell signalling and in cytoskeletal modifications. The calpains (EC 3.4.22.17) are a family of Ca2+ dependent cysteine proteases. Zongchao Jia, Qilu Ye, Peter L. Davies and John S. Elce, Department of Biochemistry, Queen’s University, Kingston, Ontario, K7L 3N6 Canada

The calpains (EC 3.4.22.17) are a family of Ca2+ dependent cysteine proteases found in the cytosol of animal cells. Their precise physiological role is uncertain, however it is likely that they are involved in cell signalling and in cytoskeletal modifications. The ubiquitous calpains have a large catalytic subunit (80 kDa) composed of 4 domains, and a small regulatory subunit (30 kDa) composed of 2 domains. The enzymes are activated by Ca2+, and then undergo autolysis. In order to understand the structural basis for Ca2+ dependent calpain activation and provide a molecular explanation for autolysis, we are interested in determining the structure of calpain II by X-ray crystallography. To avoid autolysis and oxidation which present great problems during recombinant protein production and crystallization, an inactive C105S active-site...